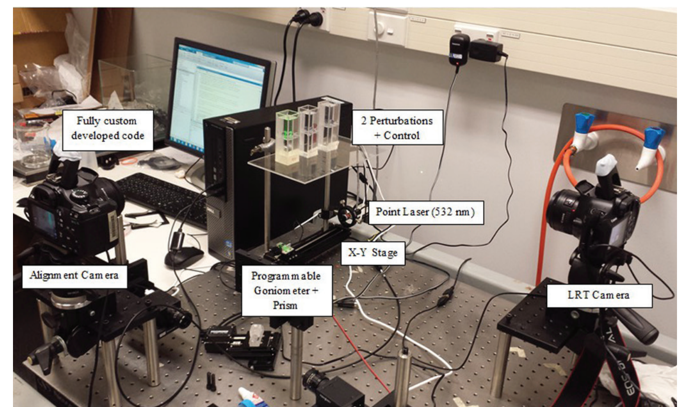
523 Lens physiology and homeostasis
Thursday, May 05, 2016 11:00 AM–12:45 PM
609 Paper Session
Program #/Board # Range: 6092–6098
Organizing Section: Lens

Program Number: 6092
Presentation Time: 11:00 AM–11:15 AM
Solute delivery to the core of the bovine lens is driven by the lens circulation system
Paul J. Donaldson[1, 2], Ehsan Vaghefi[2]. [1]School of Medical Sciences, University of Auckland, Auckland, New Zealand; [2]School of Optometry, University of Auckland, Auckland, New Zealand.
Purpose: To utilize T1-weight magnetic resonance imaging (MRI) of contrast agent penetration into the lens to test the hypothesis that the lens microcirculation system delivers solutes to the lens core faster than would occur by passive diffusion alone.
Methods: A lens of a pair of bovine lenses was organ cultured in Artificial Aqueous Humor (AAH), while the other lens was cultured in either AAH-High-K^+, or AAH + 0.1mM Ouabain for 4 hours, in the presence of MRI contrast agents of varying molecular size (GadoSpinD, 17,000g/mol; GadospinF, 1,300g/mol; FeraSpinFS, 10nm). The time course of contrast agent penetration into the lens in the different culture conditions was visualised by T1-weighted imaging utilising a 4.7T high-field small animal magnet[1]. Penetration rates of reagents were extracted and compared to rates of passive diffusion calculated by a 1D model of diffusion.
Results: Penetration of all contrast agents in the outer cortex of the lens was observed in lenses incubated in AAH, but only the lower molecular weight tracers (GadoSpinF and FeraSpinXS) were detected in the core of the lens. The pattern of GadoSpinF and FeraSpinXS penetration revealed two regions of contrast enhancement in the outer cortex and core, which were separated by a zone in the inner cortex from which the delivery of the contrast agents was restricted. The rate of delivery of GadospinF and FeraSpinXS to the core of the lens was calculated to be significantly faster by a factor of 8 than what could be achieved by passive diffusion alone. Furthermore the delivery of GadospinF and FeraSpinXS was abolished by the incubation of lenses in the presence of AAH-High-K^+ or AAH + 0.1mM Ouabain, two conditions that are known to inhibit the lens circulation system by depolarising the lens potential and blocking the Na^+ pump, respectively.
Conclusions: Our results show that the lens circulation system delivers small solutes to the lens core at a rate that is faster than would be predicted by passive diffusion alone. The extracellular pathway used to delivery solutes to the core appears to be associated with the sutures and exhibits a size selectivity that restricts the delivery of large molecules to the core. Our results support earlier work that shows an extracellular diffusion barrier exists in the inner cortex that divides the lens into two compartments.
(1) Vaghefi et al. Am J Physiol Regul Integr Comp Physiol, 302: R1250–1259, 2012.
Commercial Relationships: Paul J. Donaldson, None; **Ehsan Vaghefi**, None
Support: Health Research Council of New Zealand

Program Number: 6093
Presentation Time: 11:15 AM–11:30 AM
Monitoring the optical changes of the lens in real time under physiological perturbations
Chen/Peter Qiu[1], Paul J. Donaldson[1], Ehsan Vaghefi[2]. [1]Physiology, University of Auckland, Auckland, New Zealand; [2]Optometry, University of Auckland, Auckland, New Zealand.
Purpose: Develop a laser ray tracing (LRT) system to monitor in real time how changes to the cellular physiology of organ cultured bovine lenses alters the gradient of refractive index (GRIN) and lens geometry, and how changes to these key parameters impact the overall optical properties of the lens and bovine eye.
Methods: Bovine lenses were organ cultured in three separate chambers that contained Artificial Aqueous Humor (AAH), AAH+high K^+, and AAH+Ouabain (1.0 mM). LRT was performed on all three lenses using a fully custom built and coded system. Images of the passage of the laser light through the lens was recorded using two cameras orthogonal to each other, and the resultant data was analysed using an established tomography based method that used deflection angles and exterior ray paths to calculate the GRIN[1]. Changes in lens shape in the meridional plane were also extracted using an original image processing routine. These key optical parameters were implemented in a ZEMAX model of the bovine eye to quantify what effect each physiological perturbation had on lens power, spherical aberration, focal length and overall vision quality.
Results: Preliminary results showed that ray deflection angles and radius of curvature both increased when the lenses were incubated in AAH+Ouabain and AAH+High-K^+. Both conditions decreased the refractive index in the outer cortex (1.38 to 1.36), while at the core AAH+Ouabain increased (1.44 to 1.46) and AAH+High K^+ decreased (1.44 to 1.43) the refractive index, respectively. These changes to the optical parameters of the lens produced an increase in overall refractive power of the lens, with changes in geometry contributing primarily to the shift in the optical power, while changes in the GRIN were primarily responsible for a shift towards positive spherical aberration.
Conclusions: An automated LRT system has been developed that allows up to three lenses to be sequentially monitored in real time, enabling the effects of perturbations to their cellular physiology to be linked to changes in their optical properties. LRT is a powerful alternative to previous approaches that measure the optical properties of the lens due to lower cost, improved ex vivo control and better temporal and spatial resolution.
1. Vazquez. D et al, the Optical Society of America, 2551-2565, 2006
Acknowledgements; this work was supported by the Marsden Fund of New Zealand



Real time ray-tracing system rig
Commercial Relationships: Chen/Peter Qiu, None; **Paul J. Donaldson**, None; **Ehsan Vaghefi**, None
Support: Marsden Fund of New Zealand

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.

Program Number: 6094
Presentation Time: 11:30 AM–11:45 AM
Glutathione release from the rat lens: implications for overall ocular health
Julie Lim, Ankita Umapathy, Paul J. Donaldson. Dept Physiology, School of Medical Sciences, NZ National Eye Centre, University of Auckland, Auckland, New Zealand.
Purpose: To demonstrate glutathione (GSH) efflux from the rat lens and to identify and functionally characterise the molecular pathways involved.
Methods: Rat lenses were cultured in isosmotic AAH for varying amounts of time and GSH and GSSG (oxidised GSH) released into the media and retained in the lens measured. Lenses were cultured in the absence or presence of acivicin, a γ-glutamyl transpeptidase inhibitor, and GSH/GSSG measured. RT-PCR, western blotting and immunohistochemistry was used to screen and localise GSH efflux transporters which included members of the Multidrug Resistance Associated Proteins (Mrps). To test the involvement of Mrps in GSH release, lenses were cultured in the presence or absence of MK571, an Mrp-specific inhibitor, and GSH/GSSG measured. Finally, lenses were cultured in the presence of low (~10μM) or high (~70μM) levels of H_2O_2 and GSH/GSSG measured in the media and lenses.
Results: The lens is able to release basal levels of GSH (~125mM) under isosmotic conditions. GSH efflux was elevated in the presence of acivicin (~56% increase) indicating that GSH released from the lens undergoes some breakdown into precursor amino acids. GSH efflux was inhibited in the presence of MK571 (~73% inhibition), suggesting GSH release to be primarily mediated by Mrp transporters. Molecular screening identified Mrp1 and Mrp5 to be the most likely isoforms to mediate GSH release from the lens. In the presence of H_2O_2, the release of total GSH was increased (~2 fold increase) and this increase was driven by an increase in GSSG rather than GSH release.
Conclusions: The lens is able to release basal levels of GSH suggesting that it may contribute to maintaining GSH levels in the aqueous humour as well as providing amino acids for subsequent GSH synthesis to nearby tissues. During periods of oxidative stress, the lens releases GSSG which inadvertently may provide protection against oxidative stress to the corneal endothelium, which can utilise GSSG to regenerate GSH. Removal of the lens may reduce the availability of GSH, GSH precursor amino acids and GSSG to other anterior ocular tissues increasing their susceptibility to oxidative damage and the development of secondary ocular pathologies.

Commercial Relationships: Julie Lim, None; **Ankita Umapathy**, None; **Paul J. Donaldson**, None
Support: Sir Charles Hercus Health Research Fellowship, University of Auckland Doctoral Scholarship and the HOPE Foundation for Research on Ageing

Program Number: 6095
Presentation Time: 11:45 AM–12:00 PM
The Source of Mouse Lens Glutathione in the Absence of *De Novo* Synthesis
Jeremy Whitson[1], Catherine Doller[3], David Sell[1], Vincent M. Monnier[1, 2], Xingjun Fan[1]. [1]Pathology, Case Western Reserve University, Cleveland, OH; [2]Biochemistry, Case Western Reserve University, Cleveland, OH; [3]Medicine & Ophthalmology, Case Western Reserve University, Cleveland, OH.
Purpose: LEGSKO mouse lenses lack the enzyme GCLC and are unable to synthesize glutathione (GSH) but still to maintain millimolar concentrations of GSH through a yet undescribed transport mechanism. We generated new methods to study GSH transport *ex vivo* and *in vivo* to determine the path that GSH takes from the blood to the lens and the mechanisms involved in lens GSH uptake.
Methods: Endogenous GSH content of mammalian lenses, aqueous humor, and vitreous humor, as well as *ex vivo* and *in vivo* GSH-(*glycine*-$^{13}C_2$,^{15}N) transport were measured using LC-MS/MS. *In vivo* GSH transport was determined by catheterizing the carotid artery of mice and perfusing with labeled-GSH. The fluorescent conjugate GS-bimane was used to image GSH transport *in vivo*.
Results: Mice were found to have 1.1 +/- 0.3 mM GSH and 19.9 +/- 6 uM GSH in their vitreous and aqueous humors, respectively. *Ex vivo* lens GSH uptake measurements revealed that uptake could be measured from the lens anterior, but only at substrate concentrations above 5-fold those found physiologically. Conversely, uptake did occur rapidly when GSH-(*glycine*-$^{13}C_2$,^{15}N) was given at concentrations of 1 mM or above in cultured lenses. LEGSKO lens and GSH concentration was determined to be 1.1 +/- 0.2 mM, implying an equilibration between the vitreous and lens GSH pools. This was further indicated by measurements which show that lens and vitreous GSH content decrease equally when LEGSKO mice are treated with BSO, a specific inhibitor of GCLC. *In vivo* perfusion experiments revealed that GSH-(*glycine*-$^{13}C_2$,^{15}N), circulating at a concentration of 200 uM, accumulates at an initial rate of ~1.5 uM/min in vitreous but enters the aqueous at a rate of ~80 nM/min. Furthermore, aqueous GSH-(*glycine*-$^{13}C_2$,^{15}N) was only detectable after >2 uM had already accumulated in the lens. Preliminary *in vivo* imaging of GS-B transport in mouse eyes further confirms that circulating GSH travels to, and accumulates in, the lens and will be used to determine the tissues involved in the transport.
Conclusions: Mouse vitreous has high GSH content that appears to equilibrate with LEGSKO lenses and provide their high residual GSH concentration. *In vivo* GSH transport experiments indicate that circulating GSH rapidly enters the vitreous. This is being analyzed in greater detail by imaging the transport of GS-bimane.
Commercial Relationships: Jeremy Whitson, None; **Catherine Doller**, None; **David Sell**, None; **Vincent M. Monnier**, None; **Xingjun Fan**, None
Support: NIH Grant EY07099, NIH Grant EY024553, Institutional Training Grant EY007157

Program Number: 6096
Presentation Time: 12:00 PM–12:15 PM
Qualitative and quantitative analysis of AQP5 modifications in lens fiber cells
Zhen Wang, Kevin L. Schey. Biochemistry, Vanderbilt University, Nashville, TN.
Purpose: Lens fiber cells have two transmembrane water channels: highly abundant, low water permeability AQP0 and low abundance, high water permeability AQP5. AQP5 was found to relocate to plasma membrane from cytoplasm during fiber cell differentiation. Membrane trafficking of AQP5 could be regulated by multiple mechanisms and phosphorylation through PKA activity has been identified as one of the mechanisms. The purpose of this study is to identify new modifications that could play a role in AQP5 membrane trafficking and to quantify the level of modifications in different lens regions.
Methods: Human or bovine lens membrane fractions were prepared by removing water-soluble fraction and urea-soluble fraction. The urea insoluble fraction was either separated by SDS-PAGE followed by in-gel digestion or directly digested by trypsin, chymotrypsin or Lys C in 50 mM Tris-HCl (pH 8.0). The peptides were extracted from the gel or membrane pellets after digestion and analyzed by LC-MS/MS. An acyl-biotin exchange experiment was done to

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.

prescreen cysteine palmitoylation. A pseudo-MRM method was used for quantifying the level of modifications in different lens regions.
Results: Phosphorylation on T259 was detected in both bovine and human lens. The level of phosphorylation is high in the outer cortex region and dramatically decreases in the inner cortex and outer nucleus region. Phosphorylation was further decreased in the inner nucleus region. An acyl-biotin exchange experiment suggested AQP5 is palmitoylated. Palmitoylation of AQP5 was confirmed by direct detection of palmitoylation on cysteine 6. Palmitoylation was detected in the cortex region and dramatically decreased in the nucleus region.
Conclusions: In addition to phosphorylation, AQP5 also undergoes cysteine palmitoylation. Considering the important role of palmitoylation in membrane trafficking and protein subcellular localization, palmitoylation of cysteine 6 in AQP5 could be one of multiple mechanisms involved in AQP5 membrane trafficking.
Commercial Relationships: Zhen Wang, None; **Kevin L. Schey**
Support: NIH grant EY-13462

Program Number: 6097
Presentation Time: 12:15 PM–12:30 PM
Lens Connexin Hemichannels are Responsive to Mechanical Stimulation and Oxidative Stress, and Protect Cell against Oxidative Stress
Wen Shi, Manuel A. Riquelme, Sumin Gu, Jean X. Jiang. Biochemistry, University of Texas Health Science Center, San Antonio, TX.
Purpose: Gap junctions formed by connexins play important roles in lens homeostasis and transparency. In addition to their roles in forming gap junctions, connexins form hemichannels in lens fibers. However, the physiological importance of these lens connexin hemichannels remains largely unknown.
Methods: Lens primary cell cultures were prepared using lens at embryonic day-11 and cultured up to 15 days with the gradual formation of lentoid bodies, an indicator of lens cell differentiation. High titer recombinant retroviruses containing vehicle, chick Cx50 or Cx46, were prepared and used to infect chick embryonic fibroblast (CEF) cells and lens primary cell culture. The cells were treated with H_2O_2 at 300 μM for 20 min to 2 hrs or were subject to mechanical stimulation through fluid flow shear stress (FFSS) at 8 dynes/cm^2 for 15 min using a parallel flow chamber. Hemichannel activity was studied using dye uptake assay with ethidium bromide (EtBr) dye. Cell viability was assayed with annexin V and propidium iodide (PI) labeling.
Results: Hemichannels formed by either Cx50 or Cx46 were open in response to the treatment with H_2O_2 or FFSS. The results of dye uptake assay showed that lentoids bodies in lens primary culture were highly sensitive to H_2O_2 and FFSS, and hemichannel activity was inhibited by a connexin channel blocker carbenoxolone. Interestingly, this activity was significantly inhibited by two dominant negative mutants of Cx50; Cx50P88S which inhibits both gap junctions and hemichannels and Cx50H156N which only inhibits hemichannels, not gap junctions, implying the role of hemichannels. H_2O_2 treatment also caused the opening of hemichannels in cultured epithelial cells, but these dominant negative mutants failed to inhibit these hemichannel activities, suggesting that epithelial hemichannels are unlikely formed by Cx50 in epithelial cells. The treatment of H_2O_2 increased the numbers of cells under apoptosis and this increase was augmented in cells expressing these two dominant negative mutants.
Conclusions: These results show that both oxidative stress and mechanical stimulation activate connexin hemichannels in the lens. Functional connexin hemichannels are likely to play a cell protective role against oxidative damage in the lens.
Commercial Relationships: Wen Shi, None; **Manuel A. Riquelme**, None; **Sumin Gu**, None; **Jean X. Jiang**, None

Program Number: 6098
Presentation Time: 12:30 PM–12:45 PM
AQP0 AND BEADED FILAMENT PROTEINS ARE CRITICAL FOR OCULAR LENS BIOMECHANICS
Kulandaiappan Varadaraj[1, 2], Richard Mathias[1, 2], Paul G. FitzGerald[4], Alan Shiels[3], Nikol Palomino[1], Sindhu S. Kumari[1]. [1]Physiology and Biophysics, Stony Brook University, Stony Brook, NY; [2]SUNY Eye Institute, Syracuse, NY; [3]School of Medicine, Washington University, St. Louis, MO; [4]Cell Biology and Human Anatomy, School of Medicine, University of California, Davis, CA.
Purpose: We showed previously that AQP0 participates in maintaining lens biomechanics (Kumari et al.,2015,462:339). The present study was undertaken to explore whether AQP0 and BF proteins (CP49 and filensin) together play a role in regulating lens stiffness and resilience which are necessary for ocular lens accommodation.
Methods: AQP0 heterozygous knockout mouse expressing CP49 and filensin (AQP0$^{+/-}$/BF$^{+/+}$), and AQP0 heterozygous knockout without BF proteins (AQP0$^{+/-}$/BF$^{-/-}$) were developed in C57BL/6J strain. Genomic PCR, immunostaining and Western blotting were performed to study the expression or lack of BF proteins, as appropriate. Compression strain test was conducted to study lens stiffness and resilience. Each lens was subjected to gentle compressive strain using weighed glass coverslips. Sagittal images of lens shape changes were digitized. ImageJ software (NIH) was used to measure lens axial (A) and equatorial (E) diameters which were converted to compression-strain (T): $T = ((d-d0)/d0)$; d is 'A' or 'E' diameter at a given load; d0 is 'A' or 'E' diameter at zero load. Resilience was calculated as: $R\% = ((Ad/Ad0)*100)$; Ad is 'A' diameter after releasing coverslip load; Ad0 is 'A' diameter before applying load.
Results: Biochemical and molecular experiments confirmed the expression of BF proteins in AQP0$^{+/-}$/BF$^{+/+}$ mouse lenses and absence in AQP0$^{+/-}$/BF$^{-/-}$ lenses. Biomechanical assay revealed that loss of one allele of AQP0 significantly reduced the compressive load-bearing capacity (stiffness; P <0.001) and increased the resilience (P <0.001) compared to WT lenses. Absence of BF proteins (AQP0$^{+/-}$/BF$^{-/-}$) caused further reduction in load-bearing capacity and increase in resilience (P <0.01) compared to WT and AQP0$^{+/-}$/BF$^{+/+}$ lenses. Lens shape was significantly altered in AQP0$^{+/-}$/BF$^{+/+}$ and AQP0$^{+/-}$/BF$^{-/-}$ mice due to a higher ratio of axial: equatorial diameter.
Conclusions: Data from WT, and AQP0- and/or BF-heterozygous knockout mouse lenses suggest that AQP0 and BF proteins cooperatively maintain lens biomechanics for proper focusing. We postulate that AQP0 and BF proteins could synergistically regulate lens mechanical stiffness and resilience for accommodation. Since shape, mechanical stiffness and resilience need to be maintained for proper focusing of objects on the retina, any alteration in AQP0 functions and its interaction with other proteins such as BF proteins could contribute to presbyopia, opacity and/or cataract.
Commercial Relationships: Kulandaiappan Varadaraj; **Richard Mathias**, None; **Paul G. FitzGerald**, None; **Alan Shiels**, None; **Nikol Palomino**, None; **Sindhu S. Kumari**, None
Support: KV: EY020506, EY026155; RTM, EY06391; AS, EY012284; PGF, EY08747

These abstracts are licensed under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International License. Go to http://iovs.arvojournals.org/ to access the versions of record.